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GENETIC VARIATION OF 15 AUTOSOMAL SHORT TANDEM REPEAT (STR) LOCI IN SAMPLE OF PALESTINIAN POPULATION RESIDING IN IRAQ

[a]Mohammed Mahdi AL-Zubaidi, [*,b]Mohammed Abdul-Daim Saleh and [a]Salwa Jaber Alawedi

[a]Forensic DNA Center Research & Training, AL- Nahrain University, Iraq
[b]Department of Biology, College of Science, Diyala University, Iraq

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ABSTRACT

Allele frequencies for the 15 autosomal STR loci included in the AmpFlSTR1 Identifiler TM PCR Amplification Kit panel from Applied Biosystems (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338, D16S539) and several statistical parameters were estimated from a sample of 106 unrelated individuals**.** samples were extracted using a Prep Filer Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA), DNA quantified using Nano drop Thomson. a different number of alleles were observed with frequencies ranging between 0.005 (D8S1197- allele 9, D21S11- allele 26 and 33, D13S317- allele 15, vWA – allele 11and 20, D18S51 - allele 20and FGA- allele 28) and 0.443 (TPOX-allele 8). No significant departure from Hardy Weinberg Equilibrium (HWE) expectations were observed (a 5% significance level was taken) in the Palestinian Population residing in Iraq. The combined probability of exclusion, power of discrimination, probability of matching value for all the 15 STR loci were 0.99989468; 0.999999 and 1.0597E-18, respectively.

INTRODUCTION

Introducing of a set of Short Tandem Repeat (STR) loci as the markers induced a significant progress in this field of science (Gill *et al*., 1995; Thomson *et al*., 1999, Alonso *et al*., 2001). STR loci show variability among individuals in population and that makes these sequences important in genetic mapping, linkage analysis, identity testing in forensic cases, paternity testing, missing persons investigations, and mass disaster victim identification. In order to determine the probability of a particular genotype, population data must be gathered with a proper sample size to make an estimate of the frequency of each possible allele and genotype. The literature on STR allele frequencies contains over 1000 papers from various countries and population groups (Chakraborty, 1992). Therefore, importance of understanding the used marker heterogeneity within different populations is constantly emphasized (Thomson *et al*., 1999, Alonso *et al*., 2001; Chakraborty, 1992; Cohen, 1990). Commercial STR assays that can co-amplify as many as 16 different loci (Barni *et al*., 2007; Krenke *et al*., 2002) have become widely used in forensic DNA typing. Different number and different sets of STR loci in a different number of the individuals of southern origin were used in previous studies of Palestinian Population residing in Iraq. Namely, 15 STR loci have already been employed in a study of population of Iraq (Applied Biosystems, 2001). The aim of this work was to establish a database of the Palestinian Population residing in Iraq for forensic purposes including paternity testing. Therefore we have applied the recently introduced AmpF_STR® Identifiler™ kit that amplifies the 15 STR loci as well the amelogenin locus for gender identification. In this study we present the allele frequencies and forensic efficiency values for the 16 loci in a sample of 106 unrelated Palestinian Population residing in Iraq.

Corresponding author: Mohammed Abdul-Daim Saleh
Department of Biology, College of Science, Diyala University, Iraq

MATERIALS AND METHODS

Population: Buccal swap were collected by oral stick (Sterile Omni Swab or Sterile Foam Tipped Swabs, Whatman International Ltd., Maidstone, UK) from 106 healthy, randomly chosen from Palestinian Population residing in Iraq, samples contain both genders (male & female).

DNA extraction: samples were extracted using a Prep Filer Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA), DNA quantified using Nano drop Thomson

Table 1. Allele frequencies at 15 STR loci in Palestinian Population residing in Iraq

Allele	D13S317	TH01	D3S1358	CSF1PO	D7S820	D21S11	D8S1179
5							
6		0.208					
7		0.241		0.0141509	0.042		
8	0.127	0.193		0.009434	0.132		
9	0.042	0.245		0.014	0.160		0.005
9.3		0.094					
10	0.038	0.019		0.264	0.170		0.094
11	0.368			0.335	0.269		0.099
12	0.274			0.297	0.217		0.156
12.2							
13	0.080			0.052	0.009		0.226
13.2							
14	0.066		0.066	0.014			0.160
14.2							
15	0.005		0.198				0.212
16			0.179				0.033
16.2							
17			0.349				0.014
17.2							
18			0.189				
18.2							
19			0.019				
20							
25							
26						0.005	
27						0.019	
28						0.038	
29						0.222	
30						0.330	
30.2							
31						0.071	
31.2						0.156	
32						0.005	
32.2						0.127	
33						0.005	
33.2						0.019	
34.2						0.005	
Hom	18.87%	20.75%	22.64%	25.47%	31.13%	16.04%	18.87%
Het	81.13%	79.25%	77.36%	74.53%	68.87%	83.96%	81.13%
N	212	212	212	212	212	212	212

Table 1 (Continued)

Allele	FGA	D5S818	D18S51	TPOX	vWA	D19S433	D2S1338	D16S539
5								
6				0.014				
7								
8		0.009		0.443				0.042
9		0.061	0.014	0.127				0.255
9.3								
10		0.071		0.142				0.042
11		0.354	0.014	0.274	0.005			0.292
12		0.321	0.052			0.090		0.231
12.2								
13		0.170	0.203			0.250		0.113
13.2								
14		0.014	0.123		0.061	0.146		0.024
14.2						0.066		
15			0.189		0.085	0.231		
15.2						0.108		
16			0.146		0.292	0.038	0.061	
16.2						0.047		
17			0.090		0.302	0.024	0.212	
17.2								
18	0.019		0.137		0.151		0.113	
18.2	0.009							
19	0.099		0.019		0.099		0.179	
20	0.137		0.005		0.005		0.132	

……..Continue

21	0.175		0.009				0.014	
21.2								
22	0.108						0.038	
22.2								
23	0.123						0.137	
23.2								
24	0.132						0.080	
24.2								
25	0.118						0.009	
26	0.024						0.024	
27	0.009							
28	0.005							
29	0.042							
Hom	17.92%	27.36%	16.04%	29.25%	29.25%	16.98%	20.75%	22.64%
Het	82.08%	72.64%	83.96%	70.75%	70.75%	83.02%	79.25%	77.36%
N	212	212	212	212	212	212	212	212

Hom: observed homozigosity; Het: expected heterozigosity; N: number of alleles.

Table 2. Tests performed to determine the suitibility of markers for forensic and paternity studies

	Forensic Statistics					Paternity Statistics				
Locus	MP	PD	PIC	PE	PI	Ho	He	$\chi 2$	df	P-value
D8S1179	0.05	0.95	0.81	0.58	2.65	81.13%	83.37%	24.742	26	0.53364
D21S11	0.07	0.93	0.77	0.63	3.12	83.96%	79.41%	64.376	25	0.00003
D7S820	0.07	0.93	0.78	0.40	1.61	68.87%	80.67%	30.175	20	0.06706
CSF1PO	0.12	0.88	0.68	0.48	1.96	74.53%	72.64%	44.251	17	0.000314
D3S1358	0.09	0.91	0.73	0.52	2.21	77.36%	76.65%	26.936	15	0.029265
TH01	0.08	0.92	0.76	0.55	2.41	79.25%	79.22%	3.5598	17	0.999767
D13S317	0.09	0.91	0.73	0.58	2.65	81.13%	75.95%	24.032	20	0.241004
D16S539	0.08	0.92	0.74	0.52	2.21	77.36%	77.92%	19.381	19	0.432624
D2S1338	0.03	0.97	0.85	0.55	2.41	79.25%	86.14%	46.918	35	0.085873
D19S433	0.05	0.95	0.81	0.61	2.94	83.02%	83.43%	26.956	32	0.719873
vWA	0.08	0.92	0.75	0.43	1.71	70.75%	77.97%	30.935	19	0.04104
TPOX	0.14	0.86	0.64	0.43	1.71	70.75%	69.21%	19.781	11	0.048443
D18S51	0.04	0.96	0.84	0.63	3.12	83.96%	85.65%	162.91	34	<0.00001
D5S818	0.11	0.89	0.69	0.45	1.83	72.64%	73.41%	11.754	17	0.814772
FGA	0.03	0.97	0.87	0.59	2.79	82.08%	87.99%	97.733	40	<0.00001

MP: random match probability; PD: power of discrimination; PIC: polymorphic information content; PE: power of exclusion; PI: paternity index; Ho: observed heterozigosity; He: expected heterozigosity; $\chi 2$: Chi-square test for Hardy-Weinberg equilibrium;df: indicates degrees of freedom associated with the test P-value: probability value of Chi-square test for Hardy-Weinberg equilibrium.

Table 3. Forensic and Paternity statistical parameters of Palestinian Population residing in Iraq databases using 15 aSTR DNA markers

Sample size (n)	Palestinian Population residing in Iraq Database n=106
Combined Matching Probability (CMP)	1.0597E-18
Combined Discrimination Power (CDP)	0.999999
Combined Exclusion Probability (CEP)	0.99989468

Table 4. Results of locus-specific and global tests over loci for genetic differentiation between the Iraqi population examined and other populations

	P-values from locus-specific contingency table analyses for each comparison						
Locus	Palestinian residing in Iraq	Turkey [a]	Palestinian [b] in Gaza	Saudi [c] Arabia	Arab [d] Emirate	Oman [e]	Iran [f]
D8S1179	0.5336	0.0006	0.1412	0.3620	0.2138	0.2362	0.1314
D21S11	0.00001	0.2705	0.1574	0.9092	0.0215	0.0730	0.5622
D7S820	0.0671	0.1452	0.4238	0.2588	0.7204	0.5006	0.0280
CSF1PO	0.0003	0.3428	0.4546	0.5310	0.7335	0.3152	0.3412
D3S1358	0.0293	0.0087	0.0045	0.7102	0.0970	0.0217	0.0411
TH01	0.9998	0.7788	0.0094	0.0531	0.0550	0.0261	0.6529
D13S317	0.2410	0.0002	0.0190	0.0420	0.0077	0.0002	0.0010
D16S539	0.4326	0.1446	0.7945	0.4446	0.8835	0.3881	0.0852
D2S1338	0.0859	0.0344	0.0806	0.0182	0.0030	0.2033	0.0325
D19S433	0.7199	0.0001	0.1733	0.1094	0.0052	0.0403	0.0256
vWA	0.0410	0.0001	0.1941	0.8505	0.2186	0.4879	0.0063
TPOX	0.0484	0.1332	0.0063	0.1195	0.2361	0.0067	0.6015
D18S51	<0.00001	0.5097	0.8647	0.1278	0.8735	0.7595	0.0972
D5S818	0.8148	0.7158	0.4744	0.7014	0.3803	0.0988	0.4181
FGA	<0.00001	0.2694	0.2849	0.3000	0.3374	0.4425	0.5334

[a] Reference: [11].

[b] Reference: [12].

[c] Reference: [13].

[d] Reference: [13].

[e] Reference: [13]

. [f] Reference: [14].

PCR amplification: Fifteen autosomal STR markers (the 13 CODIS core loci and D19S433 and D2S1338) were typed along with amelogenin using the Applied Biosystems AmpFiSTR® Identifiler™ kit (3) 1±2 ng of target DNA following the protocols described in the User's Manual (applyed Biosystems). The samples were amplifyed using verity PCR System (applyed Biosystems)

Typing: Amplification products were diluted 1:15 in Hi- Di™ formamide and GS500-LIZ internal size standard (Applied Biosystems) and analyzed on the 16-capillary ABI Prism® 3130XL Genetic Analyzer. POP™-4 (Applied Biosystems) was utilized for higher resolution separations on a 36 cm array.

Data collection was performed with Data Collection v. 2.0 software (Applied Biosystems, Foster City, CA, USA) and samples were analyzed by GeneMapper1 v. 3.2 software (Applied Biosystems, Foster City, CA, USA).

RESULTS AND DISCUSSION

The observed allele frequencies for the 15 STR loci and results of forensic efficiency parameters for Palestinian Population residing in Iraq are shown in Tables 1, 2 and 3. A different number of alleles were observed with frequencies ranging between 0.005 (D8S1197- allele 9, D21S11- allele 26 and 33, D13S317- allele 15, vWA – allele 11and 20, D18S51 - allele 20and FGA- allele 28) and 0.443 (TPOX-allele 8). The highest heterozygosity is observed for D21S11 and D18S51 (83.96%) whereas the smallest heterozygosity value is obtained for D7S820 (68.87%). The loci were observed to have high discriminating power, as the power of discrimination of each loci varied from 0.86 (TPOX) to 0.97 (D2S1338 and FGA). All loci but D21S11 (0.00003), CSF1PO (0.000314), D18S51 (<0.00001) and FGA (<0.00001) met Hardy- Weinberg expectations (P > 0.05).whereas the PIC ranged from 0.64 (TPOX) to 0.87 (FGA). The combined power of discrimination for the 15 STR loci studied is 0.999999 in Table 3 which should be sufficient for the identification of any individual even for an extremely large population size. All 15 loci provide a combined probability of exclusion in non-paternity of 99.9%. The Combined Exclusion Probability for the 15 STR loci studied is (0.99989468). There were fifteen within-locus tests conducted on the Palestinian Population residing in Iraq (Table 4). No significant departure from HWE expectations were observed (a 5% significance level was taken) in the Palestinian Population residing in Iraq. The exceptions were the D21S11 (P-value =0.00001), CSF1PO (P-value =0.0003), D18S51 (P- value =<0.00001) and FGA (P-value =<0.00001) loci but when the Bonferroni procedure was used as a correction for the multiple tests performed on a population sample The allele frequencies of Palestinian Population residing in Iraq .were compared with Turkey and also with the published data of the Palestinian in Gazan, Saudi Arabiae, Arabs Emirates, Oman and Iran (Table 4). The comparison between Palestinian Population residing in Iraq and Palestinian in Gazan revealed significant differences for D21S11, CSF1PO, D18S51 and FGA using the same p-value (p-value 0.05). However, the comparison between Palestinian Population residing in Iraq and Iran, Oman and Arabs Emirates different loci D13S317, D19S433.

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